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Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures

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To cope with the increasing and less-predictable temperature forecasts under climate change, many terrestrial ectotherms will have to migrate or rely on adaptation through plastic or evolutionary means. Studies suggest that some ectotherms have a limited potential to change their upper thermal limits via evolutionary shifts, but research has mostly focused on adult life stages under laboratory conditions. Here we use replicate populations of *Drosophila melanogaster* and a nested half-sib/full-sib quantitative genetic design to estimate heritabilities and genetic variance components for egg-to-adult viability under both laboratory and semi-natural field conditions, encompassing cold, benign, and hot temperatures in two separate populations. The results demonstrated temperature-specific heritabilities and additive genetic variances for egg-to-adult viability. Heritabilities and genetic variances were higher under cold and benign compared to hot temperatures when tested under controlled laboratory conditions. Tendencies toward lower evolutionary potential at higher temperatures were also observed under seminatural conditions although the results were less clear in the field setting. Overall the results suggest that ectotherms that already experience temperatures close to their upper thermal tolerance limits have a restricted capacity to adapt to higher temperatures by evolutionary means.

KEY WORDS: **Additive genetic variance, climate change, covariance, egg-to-adult viability, evolutionary constraints, thermal adaptation.**

Stressful temperatures may affect both survival and post-stress reproduction of animals (Hoffmann and Parsons 1991; Kingsolver et al. 2011) and temperatures impinges strongly on the distribution of many ectotherms (Addo-Bediako et al. 2000; Kellermann et al. 2012a,b; Sunday et al. 2012; Araujo et al. 2013). As a consequence of ongoing climate change, the ability to adapt to increasing and more-variable temperatures is likely to impact the future abundance and distribution of species. This will be particularly relevant for those species/populations living near their thermal limits (Huey et al. 2012; Hoffmann et al. 2013; Overgaard et al. 2014) unless extensive behavioral thermoregulation can mitigate the effects of climate warming (Huey and Pascual 2009; Huey and Tewksbury 2009; Rego et al. 2010; Huey et al. 2012). Populations of species currently restricted by

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their thermal environment will therefore have to either adapt or migrate to avoid extinction given the predicted 2–6°C increase in average environmental temperature in the 21st century (IPCC 2013). Because ongoing habitat loss and barriers in the landscape limit the migration potential of many species, evolutionary shifts and plastic responses may be particularly important for dealing with ensuing climate change (Hoffmann and Sgrò 2011).

Plastic changes in morphological or physiological traits induced by acclimation and hardening treatments can significantly increase cold and heat resistance (Hoffmann et al. 2003; Chown and Nicolson 2004), and under some circumstances these responses can enable individuals to cope with thermal conditions that would otherwise be stressful or even lethal (Levins 1969; Huey et al. 1999; Kristensen et al. 2008). The physiological changes induced by exposure to suboptimal temperatures have been widely studied in insects (e.g., Zachariassen 1985; Lee et al. 1987; Hoffmann et al. 2003; Sørensen et al. 2003; Chown and Terblanche 2007). Despite the potential benefits associated with these acclimation responses, they affect upper thermal limits much less than lower thermal limits (Chown and Nicolson 2004; Overgaard et al. 2011; Alford et al. 2012). Thus, effects of heat acclimation typically increase heat resistance much less than cold acclimation increases cold resistance. Across several *Drosophila* species, developmental temperatures altered cold limits by 2–4°C, but heat limits by <1°C (Overgaard et al. 2011). Such a pattern has been demonstrated for many insects (Chown 2001; Chown and Nicolson 2004). Because plasticity of heat tolerance seems to be constrained it is unlikely to rescue populations living close to their upper thermal limit. In addition, plastic responses may be maladaptive if temperatures fluctuate and change in an unpredictable pattern (Loeschcke and Hoffmann 2007; Kristensen et al. 2008; Chidawanyika and Terblanche 2011). Finally it seems that heat tolerance of insects is far more similar between species than cold tolerance (Kellermann et al. 2012a,b). This could be due to a strong phylogenetic signal for upper thermal limits, but could also reflect different selective pressures for heat and cold resistance, respectively (Hoffmann et al. 2013).

Evolutionary responses require the presence of adaptive genetic variation in the traits under selection. Many studies on drosophilids as well as other ectotherms suggest that populations can adapt to environmental change (including warmer temperatures) through evolutionary means (Hoffmann 2010; Mukuka et al. 2010; Hoffmann et al. 2013; van Heerwaarden and Sgrò 2013; Blackburn et al. 2014). However, it has recently been suggested that evolution in some ectotherms in response to changing thermal conditions may be constrained due to low levels of adaptive genetic variation (Kellermann et al. 2009; Mitchell and Hoffmann 2010; Huertas et al. 2011; Kelly et al. 2012, 2013). These results challenge a commonly held view that all traits can be readily changed through selection (e.g., Hill and Zhang 2004; Walsh and Blows 2009). If applicable more broadly, they suggest a limited scope for evolutionary shifts to mitigate detrimental effects of climatic change.

The majority of studies investigating the ability of populations to respond to selection are performed in the laboratory, under conditions that are rarely ecologically relevant (see references in Hoffmann et al. 2013). Moreover many studies consider only a specific life stage of the organism (Kingsolver et al. 2011), whereas in nature environmental conditions vary and multiple life stages are exposed to this variation and these selection pressures. The genetic variance of physiological traits can change with environmental conditions and life stages (Hoffmann and Parsons 1991) and it is therefore important to characterize variation under those conditions in which selection will occur.

To address these issues, we examined the evolutionary capacity of a fitness-related trait, egg-to-adult viability, under ecologically relevant conditions. Specifically, we considered genetic and environmental variance components and heritabilities for egg-to-adult viability in two populations of *D. melanogaster* from tropical Queensland and temperate Victoria, Australia. We used a nested half-sib/full-sib quantitative genetic breeding design and generalized linear mixed models to investigate the genetic architecture of egg-to-adult viability under low-, benign-, and high-temperature conditions. These experiments were conducted in the laboratory and under seminatural field conditions. To provide support for the results from the quantitative genetic experiments, we performed a selection experiment under comparable conditions. The combined approach has previously been used successfully and selection experiments are proposed to be efficient in showing constraints due to a lack of variance (van Heerwaarden et al. 2008; Conner 2012). Results suggested that the evolutionary potential of egg-to-adult viability is temperature specific and lower at hot compared to benign and cold temperatures.

Materials and Methods

FLIES

The mass-bred *D. melanogaster* populations were initiated from flies collected in Australia in Innisfail, Queensland (latitude 17.52°S), and Melbourne, Victoria (latitude 37.77°S). Each of the populations was established by combining approximately 30 isofemale lines (each line established by one female mated in the field) collected in the field in March to April 2008. Populations were collected as close to sea level as possible (at elevations < 100 m). After two generations of laboratory culture, these isofemale lines were used to initiate the two mass-bred populations investigated here. After pooling the isofemale lines, which occurred for both populations simultaneously, the populations were maintained in high numbers ($N > 1000$) for 12 to 32

Table 1. Details of experiment performed in the laboratory.

	Cold	Benign	Hot
Temperature regime	15°C: 07:00–01:00 6°C: 01:00–07:00	24°C constant	34°C: 11:00–21:00 20°C: 21:00–11:00
Mean (°C)	12.8	24	25.6
Duration of experiment (days)	48	15	17
Place and time	Aarhus, Denmark; September–October 2009	Aarhus, Denmark; September 2009	Aarhus, Denmark; September 2009
Generations since establishing populations	32	32	32
Melbourne (temperate) population			
Number of eggs	4840	4720	4872
Number of vials	605	590	609
Viability (SE)	0.71 ± 0.01	0.76 ± 0.01	0.38 ± 0.01
Innisfail (tropical) population			
Number of eggs	5808	5704	5840
Number of vials	727	713	730
Viability (SE)	0.53 ± 0.01	0.72 ± 0.01	0.34 ± 0.01

Table 2. Details of experiment performed in the field.

	Cold	Benign	Hot
Mean (°C)	11.4	17.7	24.0
Max (°C)	24.0	28.0	36.5
Min (°C)	−2.0	12.0	13.0
Duration of experiment (days)	64	40	15
Place and months	Viborg, Denmark; September–November 2009	Melbourne, Australia; October–November 2008	Melbourne, Australia; January 2009
Generations since establishing populations	32	12	19
Melbourne (temperate) population			
Number of eggs	3840	12,248	13,772
Number of vials	384	1231	1678
Viability (SE)	0.30 ± 0.01	0.82 ± 0.01	0.49 ± 0.00
Innisfail (tropical) population			
Number of eggs	6600	12,988	16,088
Number of vials	661	1307	1615
Viability (SE)	0.19 ± 0.00	0.84 ± 0.00	0.53 ± 0.00

generations in the laboratory before experiments were performed (see Tables 1 and 2 for the exact number of generations). In the laboratory flies were maintained on a corn, yeast, dextrose, and agar medium at 24 ± 1°C under constant light and in a room with a relative humidity (RH) of around 50%. Despite the relatively low number of isofemale lines collected leading to a potential loss of rare alleles, we have previously (e.g., Hoffmann and Parsons 1988; Kellermann et al. 2006, 2009; Mitchell and Hoffmann 2010; van Heerwaarden and Sgrò 2013; Blackburn et al. 2014) shown that sample sizes such as these capture standing variation within the populations sampled to ensuring that robust estimates of genetic variation are obtained. Moreover, two generations of culture as isofemale lines prior to establishing mass-bred populations are not likely to lead to significant loss of genetic variation due to inbreeding, and there is also little evidence for effects of laboratory adaptation on high-temperature resistance (e.g., Griffith et al. 2005).

QUANTITATIVE GENETIC EXPERIMENTS

A nested half-sib/full-sib breeding design (Falconer and Mackay 1996) was implemented under six different thermal conditions using the two replicate populations of *D. melanogaster* (Tables 1 and

2). Larval density of the parental generation (generating parents used to produce eggs for the experimental flies) was controlled by collecting 50 eggs into each of 40 vials per population, after which vials were incubated at 24°C until emergence.

LABORATORY TREATMENTS AND DESIGN

The treatments consisted of low, benign, and high temperatures. The low- and high-thermal regimes were variable with distinct night and day temperatures. Mean temperatures in the high and low regimes were 12.8 and 25.6°C, respectively. In the benign-temperature regime the temperature was constant 24°C (Table 1). The temperatures were chosen so that developing larvae were exposed to low- and high-temperature stress as indicated by significantly lower viability compared to the benign-temperature treatment (Table 1). The thermal protocol was constructed based on data from pilot experiments to ensure that the high- and low-temperature regimes were stressful while still within the range of temperatures that could be experienced in natural hot or cold environments, respectively (Hoffmann 2010). Prior to performing the quantitative genetic experiments, including the generation in which females produced eggs that were distributed to the respective thermal environments, flies from both populations were kept at 24 ± 1°C, that is, under common garden conditions.

For the quantitative genetic experiments performed in the laboratory, 150 males (sires) and 750 females (dams) were collected under light CO_2 anesthesia from each population and used to establish nested half-sib/full-sib sire families that were tested at the three thermal conditions. Individual males were transferred to vials with 7 mL medium sprinkled with dry yeast together with five virgin females. Mating was allowed for 3 days at 24°C after which the males were removed and the females from each sire family transferred to individual vials with medium-filled teaspoons sprinkled with dry yeast. Females were allowed to lay eggs for 10 h on these spoons at 24°C. Eggs from each female were collected and transferred to replicate vials with eight eggs per vial (Table 1). We aimed at collecting eight eggs into each of the nine vials per female with three replicate vials per female distributed into each of the three thermal laboratory environments (controlled temperature cabinets). Eggs were collected over two days; on the first day a new spoon with fresh medium was inserted into each vial and females were allowed to lay eggs for a second 10-h period. On the next day egg collection was initiated again as described above. We were unable to set up nine replicate vials from all females (see Table S1 for the exact number of sires, average dams per sire, and average replicates per dam).

Because eggs collected from all nested half-sib/full-sib families were placed in all three thermal environments for development, we aimed at estimating the additive genetic correlations among egg-to-adult viability data across the three thermal laboratory environments. Eggs were collected within 16 h after being laid, and at the time of transfer to the respective temperatures no larvae were observed (see details in Table 1). Vials with developing eggs were placed into their respective cabinets and placement was changed daily to control for positional effects until all flies had eclosed. Vials were then placed at −20°C and numbers of eclosed flies in each vial scored. Thus the phenotype that we investigated (egg-to-adult viability) expresses the ability of an egg to successfully develop into an adult under the laboratory test temperatures.

FIELD TREATMENTS AND DESIGN

Vials were positioned in the shade under a cover protecting them from direct rainfall (Fig. S1). Ambient temperatures as well as light and humidity varied (Table 2), although larvae in the vials had unrestricted access to food during development. The position of vials in the field was randomized daily until all flies had eclosed. Then vials were brought back to the laboratory, and stored at −20°C until the number of eclosed flies was scored.

Field experiments were performed at different times and on different continents to enable testing under low-, benign-, and high-temperature stress (Table 2). For each of the three field experiments we used a similar procedure as described for the laboratory experiments above. Briefly, 120 density-controlled males (sires) and 600 density-controlled females (dams) were collected from each population (Innisfail and Melbourne) in the laboratory. Individual males were transferred to vials with 7-mL medium sprinkled with dry yeast together with five virgin females. Mating was allowed for 3 days at 24°C after which the males were removed and the females from each sire family transferred to individual vials with medium-filled teaspoons sprinkled with dry yeast. Females were left to lay eggs for 10 h on these spoons at 24°C in the laboratory. Eggs from each female were collected and transferred to replicate vials with 10 eggs in each vial (Table 2 and Fig. 1). We aimed to collect 10 eggs into each of the three replicate vials per female per field test temperature. To achieve this we collected eggs over two days as was done for the laboratory assessments. As for the laboratory experiments we did not succeed in obtaining three replicate vials each with 10 eggs from all females (see Table S1). The vials with eggs were then transferred to the field sites as described above (Table 2).

SELECTION EXPERIMENT—EGG-TO-ADULT VIABILITY

We used laboratory natural selection to experimentally test the results from the quantitative genetics experiments described above. Using the Innisfail population only we reared/selected flies at cold, benign, and hot temperatures for multiple generations starting 32 generations after the mass-bred populations were established. Temperatures during selection were cold (4°C from 1 a.m. to 7 a.m. and 13°C from 7 a.m. to 1 a.m.), benign (24°C constant), and hot (20°C from 9 p.m. to 11 a.m. and 35°C from 11 a.m. to

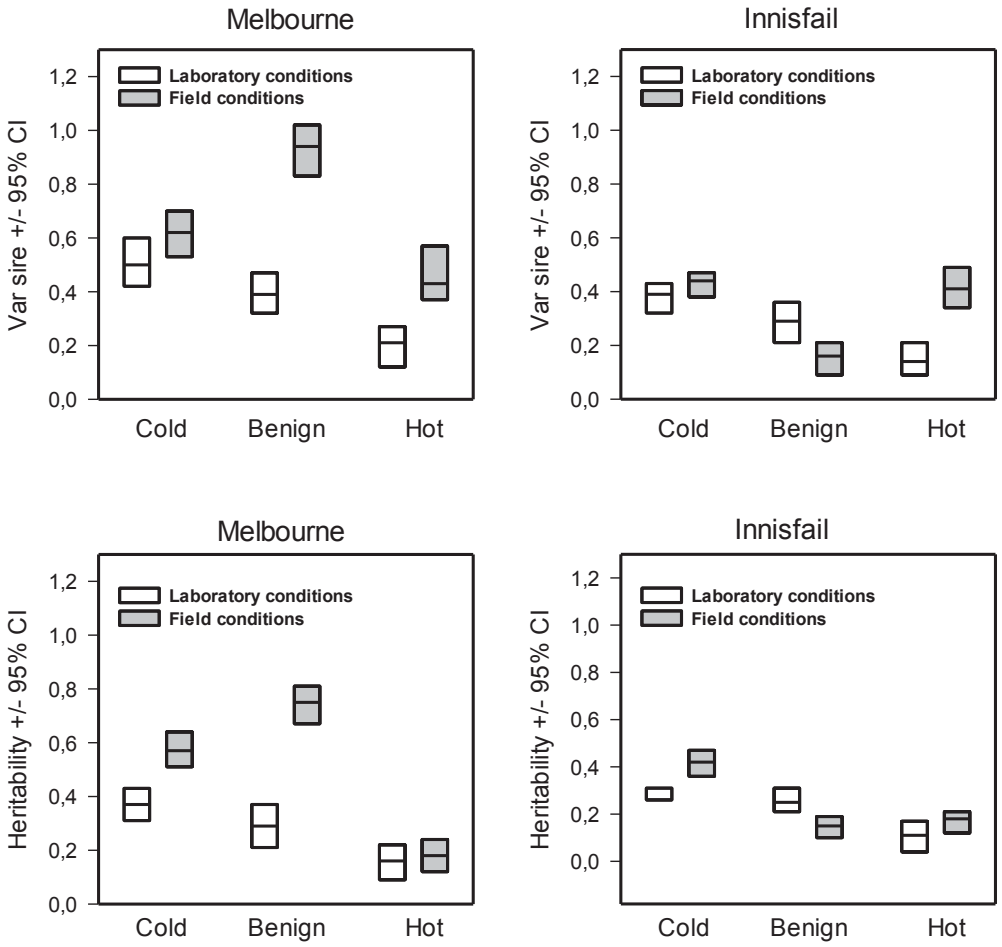

Figure 1. **Sire variances and heritabilities ± 95% CI for the *Drosophila melanogaster* Melbourne and Innisfail populations investigated at cold, benign, and hot thermal conditions in the laboratory and under seminatural conditions.**

9 p.m.). In the benign environment (control), flies were maintained at population sizes above 500 per generation distributed with approximately 100 flies in each of the five plastic round-bottom bottles (60 × 130 mm) containing 35-mL medium. Selection in the cold and hot environments was performed for every second generation. After one generation of selection, all emerging flies were transferred to 24°C and kept there for one generation. Adults from this generation were used to produce eggs that were then exposed to either the cold or hot environment in the generation that followed. The generations in a benign thermal environment were introduced to increase the population size and to reduce the risk of carry-over effects (Watson and Hoffmann 1996) that in this present study resulted in reduced adult fertility in the cold- and hot-temperature regimes, which was reversed after transfer to the benign-temperature regime.

For each generation of rearing in the cold and hot environments, 2000 eggs were distributed to each of the two thermal environments. These eggs were distributed into 40 vials (25 × 95 mm) with 7-mL medium at a density of 50 eggs per vial. On average 20 and 14% of the 2000 eggs developed successfully in the three generations of selection in the cold and hot selection environments, respectively. After each generation of selection in cold, benign, and hot environments, survivors from the 40 vials (cold and hot temperatures) or five bottles (benign temperature) were pooled to establish the next generation. We had no information on an individual fly's contribution to the next generation and were therefore not able to estimate realized heritabilities and expected selection responses.

Following three generations of selection in the hot and cold thermal environments, we reared flies from the two selection environments and the control flies in a common benign thermal environment (24°C) for two generations before testing their egg-to-adult viability in each of three thermal environments; cold, benign, and hot. The density of eggs in the vials were controlled in two generations prior to collecting eggs used in the tests by allowing 30 eggs to develop in each of 20 vials filled with 7 mL of medium. In each of the three test environments (cold, benign, and hot), 20 replicate vials from each selection regime (cold, benign,

and hot) were set up with 20 eggs per vial. Test temperatures were benign (24°C constant), cold (3°C from 01 a.m. to 07 a.m. and 13°C from 7 a.m. to 01 a.m.), and hot (20°C from 9 p.m. to 11 a.m. and 34°C from 11 a.m. to 9 p.m.). The number of flies eclosing from each vial was counted.

STATISTICAL METHODS

Effect of temperature on mean egg-to-adult viability

We analyzed mean egg-to-adult viability data from the quantitative genetic experiment using a logistic regression model:

$$logit\,(\pi_i) = \alpha + \beta \times Temperature_i,$$

where the probability of success (π) was estimated as the fraction of emerging flies from a vial. Differences in egg-to-adult viability between temperatures were assessed separately for Innisfail and Melbourne populations under field and laboratory conditions, respectively. We detected overdispersion in all models and corrected the standard errors using a quasi-generalized linear model (Zuur et al. 2009). The effect of temperature was assessed using *F*-tests. When the effect of temperature was significant, a Tukey's HSD post hoc test was performed.

Quantitative genetic analyses

In this study, survival of each egg was the observed trait of interest. The eggs that did not develop into a fly were given a score of zero and the eggs that developed into a fly were given a score of one. The analyzed phenotype was the proportion of eggs that developed into a fly from each vial. This survival trait provides phenotypes between 0 and 1 that do not have a normal distribution, meaning that statistical models assuming normality of the data were inappropriate. Instead, the data from the nested half-sib/full-sib breeding design were analyzed with generalized linear mixed models that are based on finding a relationship between the expected value of a random variable and a linear function of explanatory variables described by an appropriate link function (Tempelman 1998).The analyses were performed separately for each population and temperature treatment. Variances and covariances were estimated by applying the binomial distribution with a logit link function. In matrix notation, the threshold model was as follows:

$$\boldsymbol{\lambda} = \text{logit}(\boldsymbol{\mu} + \mathbf{Z_h h} + \mathbf{Z_s s} + \mathbf{e})$$

where $\boldsymbol{\lambda}$ is a vector of unobserved liabilities to mortality, $\boldsymbol{\mu}$ is an overall mean effect, $\mathbf{h}$ is a vector of random replicate effects, $\mathbf{s}$ is a vector of sire effects, $\mathbf{e}$ is a vector of residuals, and $\mathbf{Z_h}$ and $\mathbf{Z_s}$ are the corresponding incidence matrices. The random effects are assumed to be independently and normally distributed with means of zero. S_0 is a scalar containing the sire variance. A is a matrix with the additive genetic relationship of all animals. REP_0 is a scalar containing the replicate variance and R_0 is a scalar with the residual variance. I is the identity matrix containing as many rows and columns as there are records.

$$\text{Var}\begin{pmatrix} s \\ rep \\ e \end{pmatrix} \sim \text{N}\left(0; \begin{bmatrix} S_0 \otimes A & 0 & 0 \\ 0 & REP_0 \otimes I & 0 \\ 0 & 0 & R_0 \otimes I \end{bmatrix}\right)$$

For the laboratory data genetic covariances between traits in different populations were estimated in similar bivariate sire models. When applying this type of model S_0, REP_0 and R_0 were 2 × 2 matrices containing the variance components on the diagonal and the covariances between the traits on the off-diagonals. Heritabilities and genetic correlations were calculated using the variance or covariance of the logit link function, which implies a residual variance of $\pi^{2/3}$ (Southey et al. 2003). Using a sire model to evaluate the data, heritability estimates were calculated as $h^2 = \frac{4\sigma_s^2}{\sigma_s^2+\sigma_{rep}^2+\sigma_e^2}$. This means that variance among dams is ignored and three-fourths of the additive genetic variance ends up in the residual variance (Mrode 2005). Quantitative genetic data were analyzed in the DMU software package (Madsen and Jensen 2013). Using nonparametric bootstrap methodology sampling with replacement among sires, 95% confidence intervals (CI; Efron and Tibshirani 1986) were generated for all variance components and heritabilities using the boot package in R (R Core Team 2014) in an interface that allowed this usage. In principle the parameters and thereby confidence intervals were allowed to leave the parameter space. Significant differences between contrasts were based on nonoverlapping confidence intervals. For all confidence intervals 1000 replications were made.

Selection experiment

We analyzed egg-to-adult viability data using a logistic regression model, in which the probability of success (π) was estimated as the fraction of emerging flies from a vial. The difference in performance of selection regimes at different rearing regimes were analyzed with a full model including rearing regime, selection regime, and their interaction:

$$logit\,(\pi_i) = \alpha + \beta_1 \times Rearing_i + \beta_2 \times Selection_i + \beta_3 \times Rearing_i \times Selection_i.$$

The full model was compared to a reduced model without the interaction using a likelihood ratio test. As the interaction was significant, we analyzed each rearing regime individually with models containing selection regime as the only variable. When a likelihood ratio test revealed a significant effect of selection regime, we performed Tukey's HSD post hoc tests to analyze pairwise differences between selection regimes. The models were checked for overdispersion and there were no violations of this assumption.

Results

ASSESSMENT OF MEAN EGG-TO-ADULT VIABILITY IN THE LABORATORY AND FIELD NESTED HALF-SIB/FULL-SIB EXPERIMENTS

The analysis of egg-to-adult viability in the field and laboratory for each of the two populations investigated showed a significant effect of temperature for each population in each rearing environment ($P < 0.001$, Table 1). Post hoc tests of pairwise differences between temperatures for each population in each rearing setting revealed significant differences in all comparisons ($P < 0.001$ in all contrasts except cold vs. benign for the Melbourne population reared in the laboratory: $P < 0.01$, Tables 1 and 2).

VARIANCE COMPONENTS, HERITABILITIES, AND GENETIC CORRELATIONS ESTIMATED IN THE LABORATORY

The effect of thermal regime on sire variances and heritabilities was significant for both Melbourne and Innisfail (Fig. 1, Table S2). In general, sire variances and heritabilities were highest at the low temperature, intermediate at the benign temperature, and lowest at the high temperature across both populations (Fig. 1). Sire variances and heritabilities were significantly higher under cold compared to hot thermal environments for both populations (Fig. 1, Table S2). For the Melbourne population, the sire variance and the heritability were 2.4 and 2.3 times higher under cold compared to hot test conditions, respectively. This corresponds to, respectively, 1.9 and 2.1 SDs of the estimate (SD) apart (in each case calculated as the difference between the estimates divided by the larger of the two standard errors). For the Innisfail population, the equivalent number was 2.8 for both sire variance and heritability (respectively 2.5 and 2.8 SD apart). Heritabilities and sire variances did not differ significantly between cold and benign conditions, and benign and hot test conditions, for any of the two populations. For the Melbourne population, the variance among replicates did not differ significantly between test treatments. For the Innisfail population, the replicate variance was significantly higher in the hot compared to the benign thermal regime. Other contrasts were not significantly different (Table S2).

To investigate if egg-to-adult viability had a common genetic basis across the three different temperature treatments, we estimated the additive genetic correlations among egg-to-adult viability data across the three thermal regimes. This could only be performed for the Innisfail population. The models using the Melbourne population data did not converge, probably due to inadequate sample sizes resulting from the higher mortality experienced under the hot temperature conditions, or because of too many poorly fitting observations (several models have been tried but none of them converted). The resulting variance–covariance matrix for Melbourne, however, is positive definite. For the Innisfail population, additive genetic correlations estimated from the bivariate G-matrix of the quantitative genetic model varied from 0.64 to 0.95 with the highest correlation observed between the cold and benign thermal environments (0.95, 95% CI 0.81–1.11) and the lowest between the cold and hot thermal environments (0.64, 95% CI 0.43–0.95). The genetic correlation between the benign and hot environments was 0.94 (95% CI 0.83–1.05).

VARIANCE COMPONENTS AND HERITABILITIES ESTIMATED IN THE FIELD

The effect of thermal regime on sire variances and heritabilities was significant for both populations (Fig. 1, Table S2). For the Melbourne population, sire variances and heritabilities were significantly lower under hot conditions compared to both the benign (2.2 and 4.2 times, respectively, corresponding to 3.0 and 5.2 SD apart) and cold (1.4 and 3.2 times, respectively, corresponding to 1.4 and 3.5 SD apart) environments. In the Innisfail population, the heritabilities were significantly higher under cold compared to both benign (2.8 times corresponding to 3.4 SD apart) and hot (2.3 times corresponding to 2.8 SD apart) conditions, whereas no difference was observed between the benign and hot environments. The sire variance in the benign environment was significantly lower compared to both cold (2.8 times corresponding to 3.1 SD apart) and hot (2.6 times corresponding to 2.1 SD apart) conditions. The variance among replicates was significantly affected by test treatment in both populations. This was mainly driven by a much-higher variance in the hot environment compared to the other thermal environments (Table S2).

SELECTION EXPERIMENT

In the selection experiment we found a significant interaction between the selection regime and test environment on egg-to-adult viability ($\chi^2_4 = 42.65$, $P < 0.001$; Fig. 2, Table S3), showing that the differences in performance between cold, benign, and hot environments were dependent on selection regime. We found significant effects of selection regime in all test environments (cold: $\chi^2_2 = 24.73$, $P < 0.001$; benign: $\chi^2_2 = 9.42$, $P < 0.01$; hot: $\chi^2_2 = 9.75$, $P < 0.01$) and performed post hoc tests to assess pairwise differences within test environments. Selection at cold and hot temperatures resulted in decreased egg-to-adult viability at benign temperatures (cold vs. control: $P < 0.05$, hot vs. control: $P < 0.05$) and increased egg-to-adult viability at cold temperatures (cold vs. control: $P < 0.001$, hot vs. control: $P < 0.05$; Fig. 2). At the hot temperature, hot selected flies did not increase their egg-to-adult viability compared to controls (hot vs. control: $P = 0.97$). However, cold selection resulted in a significant decrease in egg-to-adult viability in this environment compared to controls and the hot selected lines (cold vs. control: $P < 0.05$; cold vs. hot: $P < 0.05$; Fig. 2).

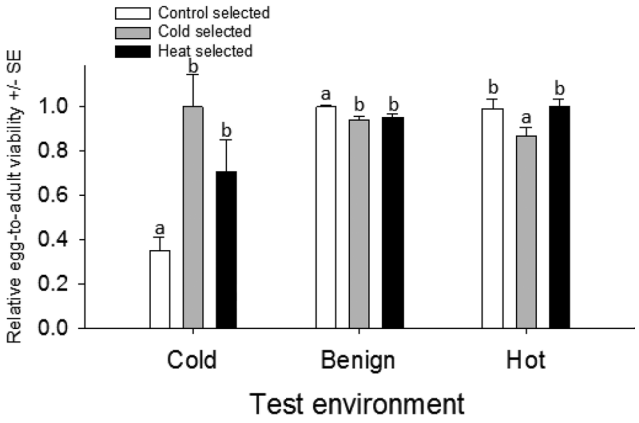

Figure 2. **Egg-to-adult viability in the *Drosophila melanogaster* population from Innisfail kept at benign control conditions or selected for increased egg-to-adult viability for three generations in a cold or hot environment. Within each test environment, egg-to-adult viability is presented relative to egg-to-adult viability of flies selected in that environment. Following selection, flies from all three thermal regimes were reared at 25°C for two generations before being tested for egg-to-adult viability in cold, benign, and hot thermal environments. Different letters within each test environment denote significant differences at the $P < 0.05$ level.**

Discussion

Climate change is expected to impose increasing selection pressures on upper thermal limits of many ectotherms. Consequently, there is increasing interest in understanding the extent and speed by which organisms can respond to thermal stress via evolution (Huey et al. 2012; Hoffmann et al. 2013). Most previous studies investigating this question have been performed under standard laboratory conditions and have primarily focused on adult life stages (Kingsolver et al. 2011; Hoffmann et al. 2013). Studies focused on ecologically relevant traits across life stages, using test conditions that better reflect thermal conditions in nature, are needed to improve our understanding of the capacity of ectotherms to adapt to increasing thermal stress in nature (Kingsolver et al. 2011; Hoffmann and Sgrò 2011; Terblanche et al. 2011; Overgaard et al. 2012; Hoffmann et al. 2013).

In the current study we addressed the scope for evolutionary change in response to thermal stress in the fitness trait egg-to-adult viability in replicate populations of *D. melanogaster*. We used a novel approach that allowed us to obtain replicated measures of sire variances and heritabilities of a fitness trait that encompasses multiple life stages under a range of conditions that are ecologically relevant. By examining a total of almost 100,000 individuals under both field and laboratory conditions, we show that sire variance and heritability estimates for egg-to-adult viability vary under different thermal conditions, and, importantly, that evolution of the trait egg-to-adult viability might be more constrained at high temperature (Tables 1 and 2 and Fig. 1). Our results support evidence from recent studies of ectotherms indicating that evolutionary potential can be compromised at high temperatures (Chown et al. 2009; Huertas et al. 2011; Kellermann et al. 2012b; Kelly et al. 2012, 2013; Araujo et al. 2013; Grigg and Buckley 2013; Schou et al. 2014).

The observation that heritabilities and sire variances are environment dependent is not novel; any text book on quantitative genetics states that the heritability of traits is environment and population specific (Falconer and Mackay 1996). Moreover, environmental stress has previously been shown to impact on heritable variation in several species including drosophilids, although these previous studies have not always demonstrated a clear pattern as to whether environmental stress increases or decreases evolutionary potential (Sgrò and Hoffmann 1998; Hoffmann and Merilä 1999; Wilson et al. 2006; Kellermann et al. 2009; Sisodia and Singh 2009). In the present study, we found significantly lower estimates of heritability under the high-thermal stress conditions compared to benign and cold conditions. The same trend was observed for sire variances although this was most clear in the studies performed in the laboratory (Fig. 1). The selection experiment showed that there was no evolutionary response in egg-to-adult viability under hot thermal conditions whereas evolution of cold resistance had occurred under selection in the cold environment (Fig. 2). Our results are therefore consistent with those of Kelly et al. (2012), who found low additive genetic variance, and a nonsignificant response to selection, for upper thermal limits in a marine copepod suggesting that evolutionary constraints of upper thermal limits could be a general feature across arthropods (see also Araujo et al. 2013).

In the present study, we examined two populations of *D. melanogaster* originating from temperate and tropical environments. Results from the two populations generally led to the same conclusion; namely that the evolutionary potential of egg-to-adult viability is higher at low compared to high temperatures. When considering the thermal conditions from where these populations originate in Australia, there is often little difference in peak summer temperatures between temperate Victoria and tropical Queensland (Hoffmann 2010; Overgaard et al. 2014). Indeed several previous studies have suggested that ectothermic species inhabiting intermediate latitudes may experience the highest temperatures (Addo-Bediako et al. 2000; Clusella-Trullas et al. 2011; Kellermann et al. 2012a,b; Overgaard et al. 2014). Based on our experiments and other results, it therefore seems likely that both temperate and tropical populations of *D. melanogaster* have a limited capacity to adapt to increasing peak temperatures via evolutionary change. However we acknowledge that our experimental design does not include the possibility that selection responses might process through the accumulation of initially rare alleles. These alleles would not have been present in our base populations initiated from 30 isofemale lines (or 120+ alleles

depending on levels of multiple mating) whereas natural populations of *Drosophila* are clearly enormous.

Our results on genetic correlations among egg-to-adult viability data in the different thermal test conditions in the Innisfail population suggest that the genetic architecture of egg-to-adult viability in the three test environments share characteristics. Thus natural or artificial selection for increased egg-to-adult viability in one thermal environment impacts on the performance in other thermal environments. However, some of the genetic variation present under cold temperatures does not appear to contribute to variation in the trait under hot conditions. Previous studies have also found that selection for increased heat resistance did not lead to unfavorable correlated responses in other fitness traits. Thus, Kelly et al. (2012, 2013) concluded that trade-offs are unlikely to explain why evolution of upper thermal limits in the tide pool copepod, *Tigriopus californicus*, is constrained and on the basis of a large comparative study investigating almost 100 *Drosophila* species, Kellermann et al. (2013) came to the same conclusion. Moreover favorable correlations were generally observed when comparing lines of *D. melanogaster* selected for a broad range of stress-resistance traits (Bubliy and Loeschcke 2005). In our study we only investigated one trait, egg-to-adult viability, and thus our design does not allow us to examine potential trade-offs with other traits. However, the significantly lower sire variance observed at high temperatures compared to benign and cold conditions for both populations in the laboratory test and for the Melbourne population in the field tests suggests that a lack of adaptive genetic variation at high and stressful temperatures (rather than trade-offs) is the most likely reason for upper thermal limits being evolutionary constrained. Thus, evolutionary changes of fitness traits at constant high temperatures as found in the hot tropics may be slow and require new mutational input (Barrett and Schluter 2008).

We can only speculate on the causes of low evolutionary potential of egg-to-adult viability at high and stressful temperatures. Populations of *D. melanogaster* from the same habitats and initiated using similar numbers of isofemale lines respond strongly to selection in other traits (e.g., Hoffmann et al. 2003; Anderson et al. 2005). In addition, this study revealed significant levels of genetic variation at low and benign temperatures. Thus a general lack of additive genetic variance in the populations is unlikely to be the explanation. It is possible that the single generation of strong selection imposed by the hot thermal regimes in this study resulted in both reduced mean and reduced additive genetic variation for egg-to-adult viability. In support of this explanation, mean egg-to-adult viability under laboratory conditions was significantly lower in the hot thermal regime compared to the cold thermal regime. However, in the field mean egg-to-adult viability was significantly higher in the hot environment compared to the cold one. Furthermore, the additive genetic variation for egg-to-adult viability was significantly higher under both the laboratory and field cold thermal regimes, indicating that a single generation of strong selection under the hot regimes did not deplete the additive genetic variance for egg-to-adult viability in the populations examined. Alternatively, it is possible that historical directional selection for optimizing fitness at high temperatures has occurred in the natural habitats of both the Melbourne and Innisfail populations. Temperatures above 40°C are regularly observed in both environments (Australian Bureau of Meteorology, www.bom.gov.au/) and frequent heat stress exposure could have resulted in the depletion of additive genetic variation (e.g., Blows and Hoffmann 1993, 2005).

Genetic drift prior to establishing the mass-bred populations might have reduced genetic variation in the populations. However, this is unlikely given the significant levels of genetic variance observed under the cold and benign temperature conditions. It is also unlikely that laboratory adaptation influenced our results, because heat resistance in *Drosophila* species does not appear to be altered when stocks are reared in the laboratory at benign temperatures for several years (Krebs et al. 2001; Griffith et al. 2005). Finally, it is possible that linkage disequilibrium prior to pooling the isofemale lines may have persisted in the mass-bred populations at the time of the experiments (King et al. 2012). However we expect the influence of this to be minor given the fact that the isofemale lines were maintained for only two generations prior to crossing to generate the mass-bred populations. Furthermore linkage disequilibrium cannot explain the different heritabilities and variance components between the different temperature regimes in the laboratory tests, which were all performed after the same number of generations of mass-bred culture (Table 1). Therefore, the low level of standing additive genetic variation under the hot thermal conditions reported in the present study are unlikely to be related to methodological problems, but rather reflect a characteristic of both populations of *D. melanogaster* examined here.

In our natural laboratory selection experiment we only exposed flies to high or low temperatures every second generation to control for carry-over effects. Similarly, two generations without selection were allowed before assessing the response to selection. This protocol meant that we were only able to select for altered viability three times across six generations and kept populations unselected for a further two generations before testing them. It is possible that a selection response would have been apparent under hot conditions if selection had been continued for more generations, with additional replication and without bouts of relaxed selection. However, based on previous results (Gilchrist and Huey 1999; Griffiths et al. 2005), we did not expect to see a change in heat resistance due to selection after such a short time, and clearly the selection procedure was sufficient to change viability under the cold conditions. Despite design limitations due to a low number of generations of selection and poor replication, these

results still provide further empirical support for the results of the nested half-sib/full-sib quantitative genetic experiments; namely that lower thermal limits are easier to change compared to upper thermal limits. The findings from the selection experiment increases our confidence in the results of the quantitative genetic experiment (see van Heerwaarden et al. [2008] for a similar example), because single measures of evolutionary potential such as heritability can be difficult to interpret on their own (Visscher et al. 2008).

In conclusion, our data suggest that tropical and temperate Australian populations of *D. melanogaster* have a reduced capacity to adapt through evolutionary changes to high- compared to low-thermal stress. This finding was found for a trait encompassing several life stages and based on results from both a selection experiment and a quantitative genetic experiment performed under ecologically relevant thermal conditions. Specifically, egg-to-adult viability did not show an evolutionary response to selection imposed by high-thermal stress, whereas selection imposed by low-temperature stress was successful in changing trait means. These differences were likely driven by different levels of additive genetic variance. The results indicate that some populations of ectotherms are constrained in their ability to shift their upper thermal limits via evolutionary changes and will face a higher risk of extinction unless they are able to migrate to habitats that provide more-suitable thermal regimes. Global change will lead to warmer but also more variable climates and in large parts of the northern hemisphere there is likely to be more snow and colder winters in the future (Cohen et al. 2012; IPCC 2013). Our data suggest that ectotherms in areas with high and constant temperatures close to their upper thermal limit have limited ability to adapt through evolutionary changes (i.e., in the tropics). However our data also suggest that temperate populations experiencing cold winters and periodically hot summers may benefit from egg-to-adult viability at cold and hot temperatures being positively genetically correlated. Thus selection for increased cold resistance during cold periods also increases performance under hot temperatures (see also Bubliy and Loeschcke 2005). Other studies have proposed that tropical populations that have evolved to cope with high and constant temperatures have reduced genetic variance caused by strong stabilizing selection (Huey and Kingsolver 1993; Bürger and Lynch 1995). Without pinpointing the mechanism, we provide further evidence that evolutionary adaptation in hot environments may be constrained.

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.1ds88.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Picture showing how vials were positioned under "field conditions."

Table S1. Number of sire families, average number of dams per sire and average number of replicate vials per dam tested in the 6 thermal regimes for the Melbourne (M) and Innisfail (I) populations, respectively.

Table S2. Mean egg-to-adult viability (out of 20 eggs) ± SE for the 3 selection regimes tested at control, hot or cold thermal conditions.

Table S3. Mean egg-to-adult viability, replicate variances, sire variances, CV_A (calculated as the square root of 4* Var sire / mean) and heritabilities ± 95% bootstrap confidence intervals for the Melbourne and Innisfail *D. melanogaster* populations investigated at cold, benign and hot thermal conditions in the lab and under semi-natural field conditions.